PATENT
38-21(13553)A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF	)	
	)	
FISCHHOFF et al.	)	GROUP ART UNIT: 1814
	)	
SERIAL NO: 08/434,105	)	EXAMINER: Hendricks
	)	
FILED: May 3, 1995	)	
	)	
TITLE: Synthetic Plant Genes and Method for Preparation	)	

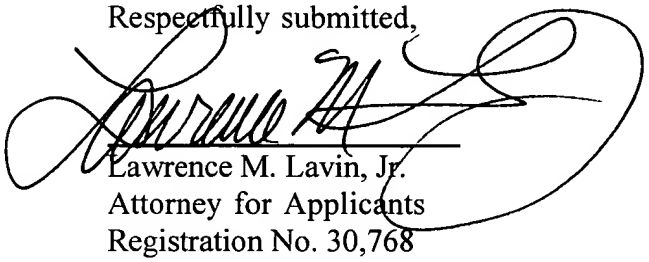
TRANSMITTAL LETTER

Enclosed herewith are the following documents:

1. Preliminary Amendment
2. Request Under 37 C.F.R. §§ 1.604 And 1.607 For An Interference And *Prima Facie* Showing Pursuant To 37 C.F.R. § 1.608
   - Exhibit 1 - '831 patent
   - Exhibit 2 - Seed & Crop Industry Article
   - Exhibit 3 - Serial No. 535,354
   - Exhibit 4 - Serial No. 848,733
3. Declaration of Frederick J. Perlak
4. Declaration of Dannette C. Ward
5. Davis (sic, Davies) Declaration
6. Declaration of Nancy L. Mathis
7. Declaration of Jeanne G. Layton
8. Declaration of Roy Fuchs

If any of the above papers are missing, it is respectfully requested that the undersigned be contacted.

Respectfully submitted,



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IN RE APPLICATION OF

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TITLE: Synthetic Plant Genes and Method for Preparation

GROUP ART UNIT: 1814

EXAMINER: Hendricks

Commissioner of Patents and Trademarks
Washington, D. C. 20231

Sir:

Transmitted herewith is an Amendment in the above-identified application.

The fee has been calculated as shown below:

CLAIMS AS AMENDED						
(1)	(2) Claims Remaining After Amendment	(3)	(4) Highest No. Previously Paid For	(5) Present Extra	(6) Rate	(7) Additional Fee
Total Claims	7*	Minus	20**	= 0	X $22.00	0
Indep. Claims	6*	Minus	3***	= 3	X $78.00	234.00
			TOTAL ADDITIONAL FEE FOR THIS AMENDMENT			$234.00

* If the entry in Column 2 is less than the entry in Column 4, write "0" in Column 5.
** If the "Highest Number Previously Paid for" is less than 20, write "20" in the space.
*** If the "highest Number Previously Paid For" is less than 3, write "3" in the space.

[X] Please charge the above calculated fee for this Amendment to Deposit Account No. 13-4125. Please charge any additional fees associated with this Amendment or credit overpayment to the above-identified Deposit Account. A triplicate copy of this sheet is enclosed.

[X] In the event an Extension of Time is required to render this paper timely filed, Applicant^ petition^ the Commissioner under 37 CFR 1.136(a) for an Extension of Time to respond for the period of time sufficient to render this paper transmitted herewith timely. The Commissioner is authorized to charge the appropriate fee for said Extension of Time to the above-identified Deposit Account.

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PATENT
38-21(13553)A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF	)	
	)	
FISCHHOFF et al.	)	GROUP ART UNIT: 1814
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SERIAL NO: 08/434,105	)	EXAMINER: Hendricks
	)	
FILED: May 3, 1995	)	
	)	
TITLE: Synthetic Plant Genes and Method for Preparation	)	

REQUEST UNDER 37 C.F.R. §§ 1.604 AND 1.607 FOR AN INTERFERENCE AND *PRIMA FACIE* SHOWING PURSUANT TO 37 C.F.R. § 1.608

NOTE: IT IS REQUESTED THAT ONCE IT IS DETERMINED WHO WILL BE MAKING THE DECISION REGARDING THIS REQUEST, THAT THE UNDERSIGNED BE CONTACTED SO THAT AN INTERVIEW CAN BE ARRANGED.

Commissioner of Patents and Trademarks

Washington, D. C. 20231

Sir:

This is a request under 37 C.F.R. §§ 1.604 and 1.607 for an interference between U.S. Patent No. 5,380,831 (the "'831" patent) issued January 10, 1995 and any continuation or divisional application from that patent and the present application.

I. REQUEST FOR INTERFERENCE UNDER RULES 604 AND 607

In accordance with Rules 604 and 607, applicants provide the following information.

A. Identification of the Patent Pursuant to 37 C.F.R. §§ 1.604(a)(2) and 1.607(a)(1)

Applicants request an interference with the '831 patent attached as Exhibit 1. While

applicants are not aware of the identity of any continuation or divisional of the '931 patent, attached as Exhibit 2 is some evidence that a continuation or divisional application of the '831 patent is pending. See the highlighted portion.

B. Proposed Count Pursuant to 37 C.F.R. §§ 1.604(a)(1) and 1.607(a)(2)

Applicants propose as the count the following:

> A method of designing a synthetic *Bacillus thuringiensis* gene to be more highly expressed in plants, comprising the steps of:
>
> analyzing the coding sequence of a gene derived from a *Bacillus thuringiensis* which encodes an insecticidal protein toxin, and
>
> modifying a portion of said coding sequence to yield a modified sequence which contains:
>
> a) a greater number of codons preferred by the intended plant host than did said coding sequence; or
>
> b) fewer putative polyadenylation signal sequences than said coding sequence; or
>
> c) both a) and b).
>
> or
>
> A synthetic gene which is derived from a *Bacillus thuringiensis* insecticidal protein toxin gene and which is more highly expressed in plants, wherein the coding sequence of said synthetic gene is modified to contain:
>
> a) a greater number of codons preferred by the intended plant host than said insecticidal protein toxin gene; or
>
> b) fewer putative polyadenylation signal sequences than said insecticidal protein toxin gene; or
>
> c) both a) and b).

The use of alternative process and product count language has been recommended in *Orikasa v. Oonishi,* 10 U.S.P.Q.2d 1996 (Comm'r Pat. 1989), particularly in those instances where the process and the product define the same patentable invention. Here, the '831 patent issued with both process and product claims, suggesting that the process and product define the same patentable invention. Moreover, the process language encompasses any process which results in the claimed product, i.e., a gene which has "greater number of codons preferred by the intended plant host . . ."

The use of alternative limitations a) and b) is appropriate because the practice of either a) or b) does not necessarily encompass the practice of the other. In other words, one can remove polyadenylation signal sequences without providing a greater number of codons preferred by the intended plant host. Likewise having a greater number of codons preferred by the intended plant host does not always eliminate polyadenylation signal sequences.

Even though the applicants are using the language of claim 1 of the '831 patent as part of the count, the applicants do not admit that claim 1 of the '831 patent defines patentable subject matter. Applicants are simply complying with the requirements of 37 C.F.R. § 1.606 that, at the initiation of the interference, the count cannot be narrower in scope than any patent or application claim corresponding to the count.

C. Identification of Claims of the '831 Patent Corresponding to the Proposed Count and Reasons Why They Correspond Pursuant to 37 C.F.R. §§ 1.607(a)(3) And 1.607(a)(4)

Claims 1-14 of the '831 patent correspond to the proposed count.

Claim 1 reads as follows:

> 1. A method of designing a synthetic Bacillus thuringiensis gene to be more highly expressed in plants, comprising the steps of:
>
> analyzing the coding sequence of a gene derived from a Bacillus thuringiensis which encodes an insecticidal protein toxin, and
>
> modifying a portion of said coding sequence to yield a modified sequence which contains a greater number of codons preferred by the intended plant host than did said coding sequence.

This claim corresponds to the count because it is the same as the method a) of the count.

A comparison of the '831 patent claim 1 and the count is below:

COUNT	CLAIM 1
A method of designing a synthetic *Bacillus thuringiensis* gene to be more highly expressed in plants, comprising the steps of:	A method of designing a synthetic *Bacillus thuringiensis* gene to be more highly expressed in plants, comprising the steps of:

analyzing the coding sequence of a gene derived from a *Bacillus thuringiensis* which encodes an insecticidal protein toxin, and	analyzing the coding sequence of a gene derived from a *Bacillus thuringiensis* which encodes an insecticidal protein toxin, and
modifying a portion of said coding sequence to yield a modified sequence which contains: a) a greater number of codons preferred by the intended plant host than did said coding sequence; or b) fewer polyadenylation signal sequences than said coding sequence; or c) both a) and b) or A synthetic gene which is derived from a *Bacillus thuringiensis* insecticidal protein toxin gene and which is more highly expressed in plants, wherein the coding sequence of said synthetic gene is modified to contain: a) a greater number of codons preferred by the intended plant host than said insecticidal protein toxin gene; or b) fewer polyadenylation signal sequences than said insecticidal protein toxin gene; or c) both a) and b).	modifying a portion of said coding sequence to yield a modified sequence which contains a greater number of codons preferred by the intended plant host than did said coding sequence.

Claims 2-10 read as follows:

2. The method of claim 1 further comprising the step of modifying a portion of said coding sequence to eliminate CUUCGG hairpins.

3. The method of claim 1 further comprising the step of modifying a portion of said coding sequence to yield CG and TA doublet avoidance indices which more closely resemble those of the intended plant host.

4. The method of claim 1 further comprising the step of modifying a portion of said coding sequence to eliminate plant polyadenylation signals.

5. The method of claim 1 further comprising the step of modifying a portion of said coding sequence to eliminate polymerase It (sic, II) termination sequences.

6. The method of claim 1 further comprising the step of modifying a portion of said coding sequence to eliminate plant consensus splice sites.

7. The method of claim 1 further comprising the step of modifying a portion of said coding sequence to yield a sequence containing a plant translation initiation sequence at the 5' end of the coding region.

8. The method of claim 4, wherein said plant polyadenylation signal is selected from the group consisting of AATAAA, AATGAA, AATAAT, AATATT, GATAAA, and AATAAG.

> 9. The method of claim 5, wherein the polymerase II termination sequence is CAN7-9AGTNNAA.
>
> 10. The method of claim 6, wherein the plant consensus splice site is selected from the group consisting of 5'=AAG:GTAAGT and 3'=TTTT(Pu)TTT(Pu)T(Pu)T(Pu)T(Pu)TGCAG:C.

Claims 2-10 correspond to the count because they depend upon claim 1 and do not claim a separately patentable invention from the count. It should be noted that claims 4 and 8 recite eliminating polyadenylation signals from a portion of the coding sequence and are the same as the methods a), b) and c) of the count.

Claims 11 and 12 read as follows:

> 11. A method of designing a synthetic Bacillus thuringiensis gene to be more highly expressed in plants, comprising the steps of: analyzing the coding sequence of a gene derived from a Bacillus thuringiensis which encodes an insecticidal protein toxin, and modifying a portion of said coding sequence to yield a modified sequence which has a frequency of codon usage which more closing resembles the frequency of codon usage of the plant in which it is to be expressed.
>
> 12. The method of claim 11, wherein the modification step comprises the substitution of at least one nucleotide in the native Bacillus thuringiensis coding sequence.

These claims correspond to the count because they are narrower than claim 1 and do not claim a separately patentable invention from the count.

Claims 13 and 14 read as follows:

> 13. A synthetic gene comprising the DNA sequence presented in FIG. 1, spanning nucleotides 1 through 1793.
>
> 14. A synthetic gene comprising the DNA sequence presented in FIG. 1, spanning nucleotides 1 through 1833.

These claims correspond to the count because they are direct products of the method of claims 1 and 11 which correspond to the count.

D. Identification of Claims of the Instant Application Corresponding to the Proposed Count and Reasons Why They Correspond Pursuant to 37 C.F.R. §§ 1.604(a)(1), (3) and 1.607(a)4

After entry of the preliminary amendment submitted with this request claims 3, 5 and 39-

43 of the above application correspond to the proposed count.

Claim 3 reads as follows:

> 3. A method for modifying a wild-type structural gene sequence which encodes an insecticidal protein of *Bacillus thuringiensis* to enhance the expression of said protein in plants which comprises:
>
> a) removing polyadenylation signals contained in said wild-type gene while retaining a sequence which encodes said protein; and
>
> b) removing ATTTA sequences contained in said wild-type gene while retaining a sequence which encodes said protein.

This claim corresponds to the count because it is the same as the method b) of the count.

Claim 5 reads as follows:

> 5. A method of Claim 3 further comprising the use of plant preferred sequences in the removal of the polyadenylation signals and ATTTA sequences.

Claim 5 corresponds to the count because it depends upon claim 3 and does not claim a separately patentable invention from the count. It should be noted that claim 5 requires plant preferred sequences, and is the same as the method a), b) and c) of the count. As such, this claim is of similar scope as the '831 patent claim 4.

Claim 39 reads as follows:

> 39. A method of designing a synthetic *Bacillus thuringiensis* gene to be more highly expressed in plants, comprising the steps of:
>
> analyzing the coding sequence of a gene derived from a *Bacillus thuringiensis* which encodes an insecticidal protein toxin, and
>
> modifying a portion of said coding sequence to yield a modified sequence which contains a greater number of codons preferred by the intended plant host than did said coding sequence and fewer plant polyadenylation signals than said coding sequence.

Claim 39 corresponds to the count because it addresses the method c) of the count as does claim 4 of the '831 patent. Applicants do not concede that the terminology of this claim 39 or claim 4 of the '831 patent conform with the requirements of 35 U.S.C. § 112, second paragraph, but add this claim merely for purposes of provoking this interference.

Claim 40 reads as follows:

> 40. A synthetic gene which is derived from a *Bacillus thuringiensis* insecticidal protein toxin gene and which is more highly expressed in plants, wherein the coding sequence of said synthetic gene is modified to contain:
>
> a) a greater number of codons preferred by the intended plant host than said insecticidal protein toxin gene; and
>
> b) fewer polyadenylation signal sequences than said insecticidal protein toxin gene.

Claim 40 corresponds to the count because it is the same as the composition c) of the count.

Claims 41-43 reproduce, in independent format, original claims 22, 23 and 24. These claims correspond to the count because they are direct products of the method of claims 3, 5, 39 and 40 which correspond to the count. They further correspond to the count for the same reason that claims 13 and 14 of the '831 patent correspond to the count.

A comparison of the application claim 3 and the count and a comparison of claim 39 and the count is below:

COUNT	**CLAIM 3**
A method of designing a synthetic *Bacillus thuringiensis* gene to be more highly expressed in plants, comprising the steps of:	3. A method for modifying a wild-type structural gene sequence which encodes an insecticidal protein of *Bacillus thuringiensis* to enhance the expression of said protein in plants which comprises:
analyzing the coding sequence of a gene derived from a *Bacillus thuringiensis* which encodes an insecticidal protein toxin, and	
modifying a portion of said coding sequence to yield a modified sequence which contains:	
a) a greater number of codons preferred by the intended plant host than did said coding sequence; or	

b)fewer polyadenylation signal sequences that said coding sequence; or	a) removing polyadenylation signals contained in said wild-type gene while retaining a sequence which encodes said protein; and b) removing ATTTA sequences contained in said wild-type gene while retaining a sequence which encodes said protein.
c) both a) and b) or A synthetic gene which is derived from a *Bacillus thuringiensis* insecticidal protein toxin gene and which is more highly expressed in plants, wherein the coding sequence of said synthetic gene is modified to contain: a) a greater number of codons preferred by the intended plant host than said insecticidal protein toxin gene; or b) fewer polyadenylation signal sequences than said insecticidal protein toxin gene; or c) both a) and b).	

COUNT	**CLAIM 39**
A method of designing a synthetic *Bacillus thuringiensis* gene to be more highly expressed in plants, comprising the steps of:	39. A method of designing a synthetic *Bacillus thuringiensis* gene to be more highly expressed in plants, comprising the steps of:
analyzing the coding sequence of a gene derived from a *Bacillus thuringiensis* which encodes an insecticidal protein toxin, and	analyzing the coding sequence of a gene derived from a *Bacillus thuringiensis* which encodes an insecticidal protein toxin, and
modifying a portion of said coding sequence to yield a modified sequence which contains: a) a greater number of codons preferred by the intended plant host than did said coding sequence; or b) fewer polyadenylation signal sequences than said coding sequence; or c) both a) and b). or	modifying a portion of said coding sequence to yield a modified sequence which contains a greater number of codons preferred by the intended plant host than did said coding sequence and fewer polyadenylation signal sequences than said coding sequence.

A synthetic gene which is derived from a *Bacillus thuringiensis* insecticidal protein toxin gene and which is more highly expressed in plants wherein the coding sequence of said synthetic gene is modified to contain:

a) a greater number of codons preferred by the intended plant host than did said insecticidal protein toxin gene; or

b) fewer polyadenylation signal sequences than said insecticidal protein toxin gene; or

c) both a) and b).

To the extent that there can be any question the instant application and the '831 patent disclose the same patentable invention, the prosecution of the '831 patent is replete with references to the instant application including statements that the instant application provides support for the patentability of the '831 patent. The patentee specifically referred to the European counterpart of the instant application, EP 0,385,962, as support for patentability of the '831 patent. See the response dated 10/28/91 (paper no. 11) at, for example, pages 16-17 and 22; the supplemental declaration of Kemp and Sutton dated 1/30/92 (paper no. 20); the preliminary amendment dated 1/28/92 (paper no. 22); the amendment dated 11/16/92 (paper no. 28) page 14 (". . . the Fischhoff et al. '962 reference discloses data which corroborates the veracity of Applicants' disclosure. . ."); the amendment dated 6/16/93 (paper no. 37), and the amendment dated 6/30/94 (paper no. 40).

E. Reasons That Claims of U.S. Serial No. 959,506 Do Not Correspond to Count

The parent application to the instant application, Serial No. 959,506 filed October 9, 1992, has pending claims directed to changes in the "B" or "240" region. Claim 43 of that application reads as follows:

> 43. A modified chimeric gene comprising a promoter which functions in plant cells operably linked to a structural coding sequence and a 3' non-translated region comprising a polyadenylation signal which functions in plants to cause the addition of polyadenylate nucleotides to the 3' end of the RNA, wherein said structural coding sequence encodes a toxin protein derived from a *Bacillus thuringiensis* protein, wherein said structural coding sequence comprises a DNA sequence which differs from the naturally occurring DNA sequence encoding said *Bacillus thuringiensis* protein and comprises the following characteristics:
>
> said naturally occurring DNA sequence comprises a region having the following

sequence:

```
TTAATTAACCAAAGAATAGAAGAATTCGCTAGGAAC
1   5   10   15   20   25   30   35
```

and said structural coding sequence has been modified, said modifications comprising at least one modification in said region selected from the group consisting of:

nucleotide 1 is a cytosine (C);

nucleotide 3 is a cytosine (C);

nucleotide 6 is a cytosine (C);

nucleotide 12 is a guanine (G);

nucleotide 18 is a cytosine (C);

nucleotide 24 is a guanine (G); and

nucleotide 36 is a thymine (T).

As discussed in the response submitted October 24, 1995 in Serial No. 959,506, this claim is patentable over the '831 patent even if the '831 patent were considered to be part of the prior art. Nothing in the '831 patent suggests or discloses either the importance of making changes in the "240" or "B" region or that changes in this region are effective to increase expression of a *B.t.* gene in plants. This subject matter provides surprising results.

37 C.F.R. § 1.601(i) states that an interference is instituted when two or more parties are claiming the same patentable invention. "Same patentable invention" is defined in 37 C.F.R. § 1.601(n) as follows:

> Invention "A" is the *same patentable invention* as an invention "B" when invention "A" is the same as (35 U.S.C. 102) or is obvious (35 U.S.C. 103) in view of invention "B" assuming invention "B" is prior art with respect to invention "A". Invention "A" is a *separate patentable invention* with respect to invention "B" when invention "A" is new (35 U.S.C. 102) and non-obvious (35 U.S.C. 103) in view of invention "B" assuming invention "B" is prior art with respect to invention "A".

Claims 43, 51, 78-81, 100-102 and 112-114 of Serial No. 959,506 are directed to subject matter specifically disclosed in the instant application that is not disclosed or suggested in the

'831 patent. Therefore, these claims are new and non-obvious over the '831 patent, define a *separate patentable invention* and should not be designated as corresponding to the count.

Likewise, claims to a method of producing a *B.t.* gene containing modifications in the "B" region or claims to *B.t.* genes containing modifications in the "B" region, such as original claims 15-21 and 26 define a *separate patentable invention* and should not be designated as corresponding to the count.

F. Application of Terms of Applicants' Claims to the Disclosure in the Application Pursuant to 37 C.F.R. § 1.607(a)(5)

As originally filed on February 24, 1989, U.S. Serial No. 315,355, filed ("the '355 application"), the great grandparent application to the instant application, presented claims 1 and 3 which read as follows:

> 1. A method for modifying a wild-type structural gene sequence which encodes an insecticidal protein of *Bacillus thuringiensis* to enhance the expression of said protein in plants which comprises:
>
> a) removing polyadenylation signals contained in said wild-type gene while retaining a sequence which encodes said protein; and
>
> b) removing ATTTA sequences contained in said wild-type gene while retaining a sequence which encodes said protein.
>
> 3. A method of Claim 2 further comprising the use of plant preferred sequences in the removal of the polyadenylation signals and ATTTA sequences.

These claims are repeated exactly in the instant application as original claims 3 and 5. Thus, these claims are clearly supported in the priority document, the '355 application, as well as the instant application. These claims, as well as language in the specification, further support newly added claims 39 and 40. See page 20, lines 13-32 of the '355 application.

Claims 41 to 43 correspond to original claims 22, 23 and 24 of the instant application, rewritten in independent form. Claims 22, 23 and 24 are supported in the '355 application where they occur as original claims 20, 21 and 22.

G. Meeting the Requirements of 35 U.S.C. § 135(b) Pursuant to 37 C.F.R. § 1.607(a)(6)

The '831 patent issued on January 10, 1995 and has not been issued more than one year. All claims pending as of the amendment attached as Exhibit 3 either were in a parent, grandparent or great-grandparent application prior to the issuance of this patent or put in by this amendment, well before January 10, 1996.

II. *PRIMA FACIE* SHOWING PURSUANT TO 37 C.F.R. § 1.608.

A. The Priority of the '831 Patent

The '831 patent is at best entitled to a priority date of September 9, 1988. Although it claims a priority to U.S. Serial No. 535,354, filed September 26, 1983 and U.S. Serial No. 848,733, filed April 4, 1986, neither the 1983 application nor the 1986 application make any reference to plant preferred codons much less the use of plant preferred codons or the removal of potential polyadenylation sequences to improve expression of the gene in plants. For the Examiner's benefit, included with this submission as Exhibits 3 and 4 are the priority applications. The subject matter of the proposed count was addressed for the first time in U.S. Serial No. 242,482, filed September 9, 1988.

B. Applicants' Can Show Reduction to Practice In the United States Prior to September 9, 1988

The present application was filed on May 3, 1995 as U.S. Serial No. 08/435,105. It is a divisional application of U.S. Serial No. 959,506, filed October 9, 1992 which was a continuation of U.S. Serial No. 476,661, filed February 12, 1990, which, in turn, was a continuation-in-part of U.S. Serial No. 315,355, filed February 24, 1989 (the '355 application).

In preparation for filing the '355 application and indeed before the September 9, 1988 filing date that the '831 patent is presumedly entitled to, applicants had produced synthetic *B.t.* genes or had others produce synthetic *B.t.* genes that contained both more plant preferred codons and fewer polyadenylation signal sequences than the wild-type or native *B.t.* gene, had inserted them into plant cells, had regenerated these plant cells into plants and had tested the plants for

resistance to insects and for expression of the *B.t.* genes[1]

The acts of applicants and others described above occurred prior to September 9, 1988 in the United States and are further described in the attached declarations. This evidences supports a *prima facie* case that applicants reduced the invention to practice prior to the '831 patent and are entitled to judgment relative to the patentee of the '831 patent for the subject matter of the proposed count.

The following is an outline of the attached declarations and documentation.

C. Summary of Declarations Submitted Pursuant to 37 C.F.R. § 1.608(b)

Dr. Fischhoff and Dr. Perlak, the inventors of the instant application, had several discussions regarding the failure of structural coding sequences from *Bacillus thuringiensis*, particularly structural coding sequences encoding the full-length protein, to express in plants and plant cells. While sequences encoding a truncated protein were expressed in plants and plant cells to some extent, these sequences were expressed at very low levels. The discussions between Dr. Fischhoff and Dr. Perlak led to their conclusion that certain sequences and the overall "A" and "T" content of the coding sequence were responsible for the failure of these genes to express or the reduced expression levels. See Exhibit 2 of the Perlak Declaration which are pages 3547889 and 3547890 of Dr. Fischhoff's notebook.

As a result of these discussions and as described in the last two paragraphs of Exhibit 2 to the Perlak Declaration two separate strategies were pursued for production of synthetic *B.t.* genes to improve the expression of these genes in plants. One was to create a completely synthetic gene. Another was to modify an existing gene by site directed mutagensis. The sequences produced by these two strategies were presented as examples in the original patent application as Figures 2 and 3, respectively. Both were produced concurrently at Monsanto by Drs. Fischhoff and Perlak.

---

[1] Apparently, the applicants for the '831 patent had not produced any synthetic genes at all as of the September 9, 1988 filing date of Serial No. 242,482. See the response dated October 31, 1991 (Paper No. 11) addressing reasons for their failure in confirming operability of the gene set out in Figure 1 of the '831 patent.

The Fischhoff notebook pages that form Exhibit 2 to the Perlak Declaration were witnessed by Dannette C. Ward. A corroborating declaration by Ward is submitted with this request.

1. Production of a Completely Synthetic B.t. Gene

a) Perlak Declaration

The Perlak notebook page attached as Exhibit 3 of the Perlak Declaration shows the sequence of a chemically synthesized gene encoding a *Bacillus thuringiensis* toxin protein (a *B.t.k.* HD-1 gene) which has been modified to reduce the occurrence of A-T rich regions and regions which induce instability or premature termination in genes, such as ATTTA sequences and polyadenylation signal sequences. This coding sequence was written out prior to September 9, 1988 and is shown in Figure 3 of the instant application and the '355 application, the priority application.

b) Davis (sic, Davies) Declaration

British Biotechnology was engaged to synthesize the the sequence shown in Exhibit 3 of the Perlak Declaration. British Biotechnology synthesized the DNA in three parts and sent the completed sequences back to Monsanto in St. Louis prior to September 9, 1988. See the Davis (sic, Davies) Declaration. .

c) Perlak Declaration (continued)

Dr. Perlak received the three parts constituting the Exhibit 3 sequence from British Biotechnology. Dr. Perlak assembled the three parts into a single DNA sequence. See Exhibit 8, page 3850033 and 3850040 of the Perlak Declaration. This sequence was then inserted into a chimeric gene construct, i.e., between a plant promoter and a plant functional polyadenylation signal sequence. The chimeric gene construct containing the sequence was then inserted into an *Agrobacterium* vector suitable for transforming plant cells. The resulting combination was identified at Monsanto as "pMON5377" which means it is a Monsanto plasmid numbered 5377. The *B.t.* sequence can then be referred to as the "pMON5377" sequence. The resulting vector containing the pMON5377 sequence was submitted to the plant transformation group for

transformation into plant cells.

In removing these A-T rich regions and regions which induce instability or premature termination, the pMON5377 sequence has also been changed from the wild type sequence to reduce the occurrence of codons which are little used in a plant. See item 3 under "possible causes" on page 3706667 of Exhibit 3 to the Perlak Declaration. Figure 3 of the present application shows both the wild-type sequence and the changes that were made that result in the pMON5377 sequence

d) The pMON5377 Synthetic Sequence Contains "A Greater Number Of Codons Preferred By The Intended Plant Host."

Figure 3 of the present application is reproduced in the Perlak Declaration at Exhibit 4, with the additional showing of the amino acids encoded. The polyadenylation signal sequences and ATTTA sequences are underlined. The codons that were changed from the wild type codons resulting in codons more often used by plants (based upon the instant application codon preference table as set out in Table V of the instant application) are marked with a "*" and are printed in red. These changes resulted in a coding sequence (the "pMON5377" sequence) that has 350 codon changes, of which 268 were codons more preferred by plants, based upon instant application's codon table, than the wild type codon it replaced. Thus, there were 186 (350 total changes with 268 "preferred" minus 82 "not preferred") more codons preferred by the intended plant host, in this instance, dicot plants, such as tomato, tobacco and cotton plants, than in the wild-type.

Alternative methods exist for measuring that the pMON5377 sequence has "a greater number of codons preferred by the intended plant host." Some of these alternative measurements are set forth in the table attached to the Perlak Declaration at Exhibit 5. In that table, columns 1 and 2 show each amino acid and the codons used to encode that amino acid.

> Column 3 shows the percent usage of those codons in dicot plants as calculated from Table V of the instant application.
>
> Columns 4 and 5 show the same percent usage as calculated in the '831 patent for dicots (column 4) and monocots (column 5).

Column 6 identifies, by symbol, those codons that meet the descriptions in columns 9-12, based upon the '831 patent's plant usage percentage for dicots (Column 4). For example, according to the '831 patent, the least common (the least plant preferred) codon (marked by a "-") for Glycine in dicots is GGG.

Column 7 shows the codon usage of the wild-type B.t. gene.

Column 8 shows the changes made as a result of the product of the synthetic gene of Exhibit 2.

Exhibit 5 of the Perlak Declaration demonstrates in column 9 that, using an arbitrary selection of 20%, there is an increase in the number of codons used more than 20% of time. Column 10 shows that there is also a decrease in the number of codons used 20% of time or less. Column 11 shows an increase in the number of most preferred codons used. Column 12 shows a decrease in the least preferred codons used. Each of these measurements is relevant to show "a greater number of codons preferred by the intended plant host."

The '831 patent provides yet another measurement of whether a synthetic *B.t.* gene contains "a greater number of codons preferred by the intended plant host." At Column 7, lines 45-51, the '831 patent provides a formula which allegedly measures the overall average deviation of the codon usage of a synthetic gene from that of a host cell. The lower this number is, the closer the codon usage of the synthetic gene is to that of a plant and, presumably, the more plant preferred codons that are present.

Applying this formula to the pMON5377 sequence shows that the pMON5377 sequence has a lower overall average deviation of the codon usage from that of a plant cell than the wild type *B.t.* sequence. Exhibit 6 shows that the wild-type *B.t.* sequence, before any change in codons, has a deviation of over .63 (see page 3 of Exhibit 6). The pMON5377 sequence, on the other hand, as demonstrated in Exhibit 7, has a deviation of about .42 (See page 3 of Exhibit 7). Thus, the pMON5377 sequence is closer to the codon usage of a plant cell than the wild type *B.t.* gene and presumptively has a greater number of codons preferred by the intended plant host.

e) Mathis and Layton Declarations.

These declarations of Monsanto employees detail the transformation of tobacco (Mathis) and tomato (Layton) plant cells with the pMON5377 *B.t.* DNA sequence. The cells were tested for the presence of the DNA sequence and then regenerated into plants. Cells and plants were then transferred to Roy Fuchs at the Monsanto Biological Sciences Department for analysis. All this work was completed in the United States prior to September 8, 1988.

2. **Production of a Partially Synthetic B.t. Gene**

a) Perlak Declaration

During the same time period as events described above, Dr. Perlak was working on a second strategy to improve the expression of *B.t.* DNA sequences in plants. This second strategy involved the preparation of a partially synthetic B.t. gene using site directed mutagensis. The first step in this process was the manufacturing of oligonucleotide sequences for use in making the desired changes. Exhibit 9 identifies the oligonucleotide sequences used to produce modified *B.t.* sequences. The summary of the location of the changes made in the *B.t.* sequences is shown on page 3706700 of Perlak Declaration Exhibit 9.

The modified *B.t.* sequences were then combined to produce one sequence which contained modifications resulting from the use of all of the oligonucleotides. The work to produce this combined sequence is shown in part on pages 3850008 and the summary of the vectors used to produce this combined sequence is shown on page 3850018 of Exhibit 9 to the Perlak Declaration. This sequence was designated pMON5360. This sequence was then inserted into a chimeric gene construct, i.e., between a plant promoter and a plant functional polyadenylation signal sequence. The sequence was then inserted into an *Agrobacterium* vector suitable for transforming plant cells and the resulting plasmid was designated pMON5370. See page 3850029 of Exhibit 9. The pMON5370 sequence is shown at Figure 2 of the instant application.

The resulting vector containing the pMON5370 sequence was submitted to the Monsanto plant transformation group for transformation into plant cells.

b) Mathis and Layton Declarations.

These declarations of Monsanto employees detail the transformation of tobacco (Mathis) and tomato (Layton) plant cells with the pMON5370 *B.t.* DNA sequence. The cells were tested for the presence of the DNA sequence and then regenerated into plants. Transformed cells and plants were then transferred to Roy Fuchs at the Monsanto Biological Sciences Department for analysis.

3. **Analysis of Synthetic *B.t.* Genes - Fuchs Declaration**

Roy Fuchs was a Senior Research Specialist in the Monsanto Biological Sciences Department. He was responsible for testing transformed plants for expression of B.t. genes and toxicity to insects. His declaration describes the testing of plant cells and plants for expression of the pMON5370 and pMON5377 *B.t.* DNA sequences as well as the testing of the toxicity of those transformed plants to insects.

Dr. Fuchs' notebook page 4086401 describes a test of leaf tissue from tobacco plant calli for the expression of *B.t.* protein from plants transformed with vectors containing *B.t.* DNA sequences identified as pMON5370 and pMON5377. The pMON5370 sequence is the partially modified sequence and the pMON5377 is the completely synthetic *B.t.* sequence. pMON9921 was used as comparison *B.t.* DNA sequences and did not contain any modifications in the DNA sequence to improve expression in a plant cell.

Fuchs' notebook page 4086402 shows the ELISA results from the test for the presence of the *B.t.* protein described on the previous notebook page. The ELISA data shows that pMON5370 and pMON5377 sequences were expressed at much higher levels than the control sequence pMON9921. As Dr. Fuchs stated on that page:

> Super! - Syn. HD1 (5377) gene expressed great for tobacco - higher than mod. HD1 (5370) which was superior to HD1 29-607 (9921). Shows that changing codons from Bt to plant preferred codons yields much higher expression. Also disrupted AT rich regions. Also, would predict mod. gene to be expressed at intermediate levels between syn. (5377) and wild type (9921). True here. Great! Should extrapolate to whole plants - tobacco, tomato,

cotton, etc. Novel approach to increase Bt. expression and expression of other poorly expressed genes in plants!

Notebook pages 4086403-4 show the Western blot analysis data on the plants containing the same *B.t.* DNA sequences. This data confirmed the results found in notebook page 4086402 that plant tissue containing the completely synthetic pMON5377 sequence expressed the *B.t.* protein at higher levels than plant tissue containing the partially modified pMON5370 sequence, which expressed the *B.t.* protein at higher levels than plant tissue containing the control sequence, pMON9921.

Fuchs notebook pages 4086405-6 show tests of toxicity of transformed tomato plants to tobacco horn worm (THW). The pMON5361 and the pMON5370 sequences were identically modified synthetic genes developed by Dr. Perlak (the distinction being in the chimeric gene promoter region, not in the *B.t.* DNA sequence). The results on these pages support Dr. Fuchs' conclusion that the modified *B.t.* DNA sequences performed better than the pMON9920 series of sequences, which contained unmodified *B.t.* DNA sequences.

Fuchs notebook pages 4086407-8 show ELISA data on the plants tested on pages 4086405-6. These test confirm a correlation between the ELISA data and the insect toxicity.

Fuchs notebook pages 4086409-10 shows tests of toxicity of transformed tobacco plants to tobacco horn worm (4086409) and tobacco bud worm (4086410). Again, this data confirms the ELISA and Western blot data that the pMON5370 sequence is highly expressed resulting in good toxicity to insects.

The data in these notebook pages demonstrates that the expression of *B.t.* genes with modifications in the coding sequence to increase plant preferred codons and eliminate AT rich regions, such as are present in polyadenylation signal sequences, dramatically improves the expression of the *B.t.* gene in plant cells.

4. Timing of the Events Described In the Declarations

All of the events described in each of the above declarations occurred before September 9, 1988 and, with the exception of the synthesis of the three pieces that made up the pMON5377

sequence by British Biotechnology (Davis Declaration), these events occurred in the United States. These events were all recorded on the attached reports and notebook records attached to the declarations prior to that date and were all dated by the record keeper earlier than September 9, 1988.

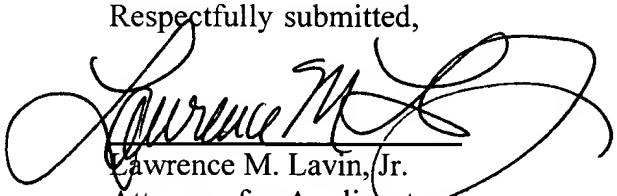
III. CONCLUSION

It is clear from the above that the '831 patent and the instant application claim the "same patentable invention" as defined in 37 C.F.R. § 1.601(n). It is also clear from the attached declarations that applicants both conceived and reduced to practice of the subject matter of the proposed count prior to September 9, 1988. Thus, applicants have established a *prima facie* case that they are entitled to judgment relative to the patentee of the '831 patent.

NOTE: IT IS REQUESTED THAT ONCE IT IS DETERMINED WHO WILL BE MAKING THE DECISION REGARDING THIS REQUEST, THAT THE UNDERSIGNED BE CONTACTED SO THAT AN INTERVIEW CAN BE ARRANGED.

In view of the above, a declaration of interference is respectfully requested.

Respectfully submitted,



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